CYTOGEOGRAPHY OF ORONTIUM AQUATICUM (ARACEAE)[1]

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The Golden Club, *Orontium aquaticum* L., constitutes a monotypic genus of subfamily Calloidae Engl., an alliance also including the genera *Symplocarpus, Lysichitum* and *Calla.* These genera are interpreted as temperate or subarctic extensions of the predominantly tropical family Araceae. All but *Orontium* are represented in eastern Asia and in North America, and thereby lend strength to the affinities existing between the floras of these regions.

Morphologically, *Orontium* is regarded as a derived, highly modified genus without any near relatives. The geographical distribution of the genus and its allies lends considerable support to this view.

Orontium aquaticum is a perennial herb possessing a deep-seated, firmly-anchored, vertical rhizome. Its distribution (Fig. 1) is restricted to the eastern United States, where it occurs on the Coastal Plain in aquatic or semiaquatic habitats such as bogs, marshes, streams and pools, and in similar habitats of the inland physiographic provinces, sometimes up to 2800 ft. in the mountains. However, lack of suitable habitats has localized its distribution in the Appalachian Highlands.

To date there has been no comprehensive morphological study of *Orontium.* Harshberger (1916), in his account of the vegetation of the New Jersey pine barrens, described and illustrated the anatomy of the leaf in considerable detail. In a meticulous study of the floral structure,

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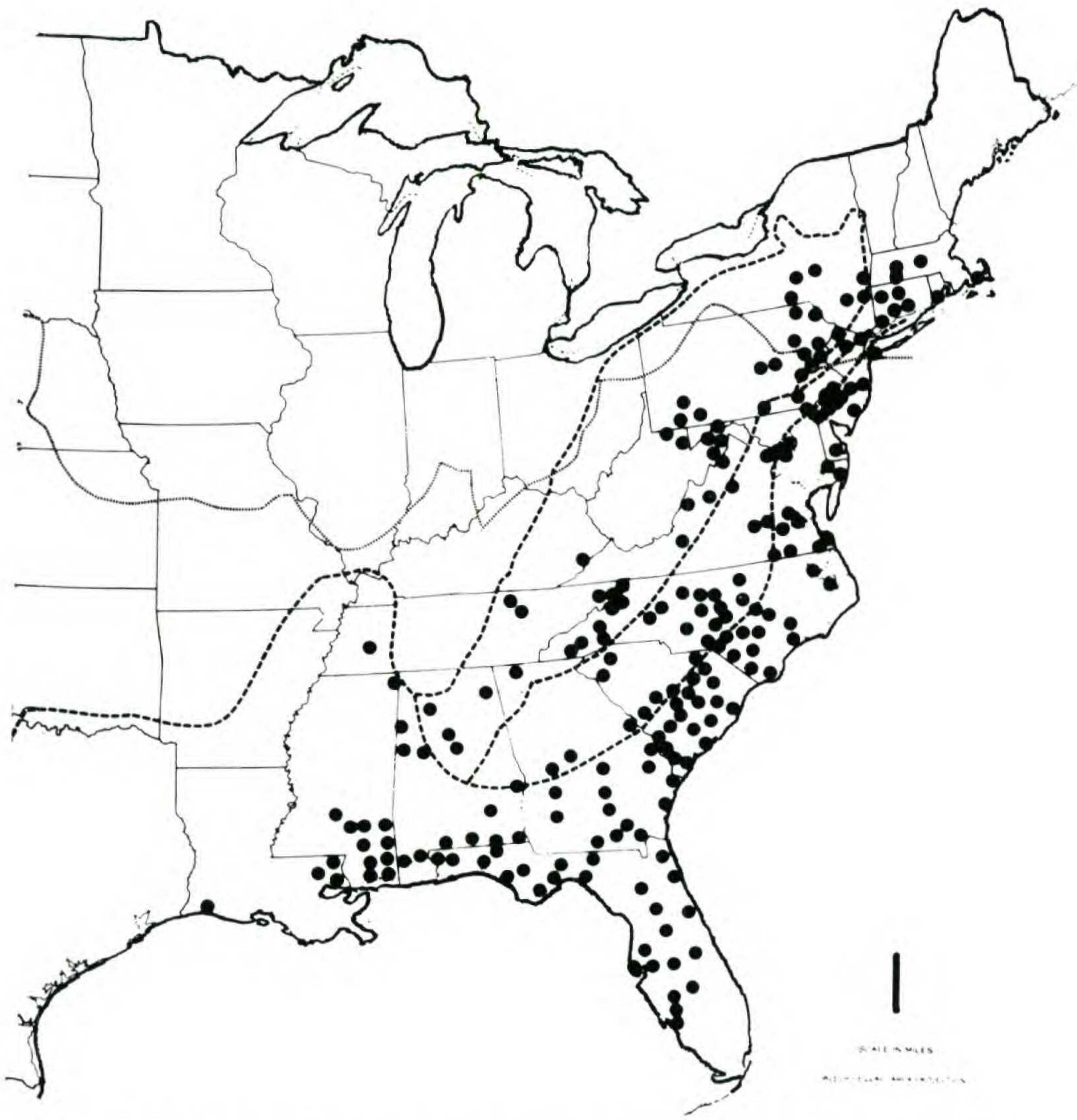

Fig. 1. Map showing distribution of *Orontium* based upon records documented by herbarium specimens. The southernmost extent of glaciation is shown by the dotted line. The heavy broken lines separate respectively the Coastal Plain, Piedmont and Appalachian highlands, including the Blue Ridge, Ridge and Valley and Appalachian Plateaus provinces.

Schaffner (1937) pointed out inaccuracies of previous descriptions and reported a wide range of variation among the flowers on a single spadix. In a taxonomic study of the temperate North American Araceae, Huttleston, (1953, unpublished) found the chromosome number of *Orontium* to be $2n=28$, a number different from that previously found by Malvesin-Fabre ($2n=24$, 1945, unpublished) see Darlington and Wylie, 1956. In the treatment of the Arales for the generic flora of the Southeastern United States, Wilson (1960) summarized the existing information concerning *Orontium,* indicating that the morphological and biological details of this plant warranted further investigation.

The present investigation was prompted by conflicting statements about the chromosome number as well as the relationship between this data and the distribution of the plant in all major physiographic provinces of the Eastern United States. It was further felt that by correlating cytological with geographical data, some insight into migrational patterns might be gained.

Through the correlation of past geological history with cytological, morphological, taxonomical and ecological evidence, it is sometimes possible to postulate an earlier distribution and possible migration route, which would explain the present distribution of *Orontium.* The Araceae are a predominantly tropical family and during the Tertiary period, when tropical conditions prevailed farther northward, a wide range of plants such as temperate conifers, deciduous hardwoods and warm-temperate plants, including araceous plants, ranged to high northern latitudes, far beyond the limits of the present tropics.

In *Symplocarpus* and *Lysichitum,* the two genera most closely related to *Orontium,* the migration route to present ranges appears to have been from the region of the Arcto-Tertiary flora of the then tropical boreal regions of western North America and eastern Asia (Rosendahl, 1911). Subsequent climatic changes forced them to migrate southward. *Symplocarpus* apparently migrated to eastern North America while *Lysichitum* remained in the West. As with *Orontium,* plants in both of these genera require a very moist or wet habitat. *Symplocarpus foetidus* (L.) Nutt., constituting a monotypic genus, occurs in two widely disjunct areas — eastern Asia and eastern United States. *Lysichitum* consists of two closely related species: *L. camtschatcense* (L.) Schott, the Asiatic one, occurs in Japan and Kamtschatka, and its American counterpart, *L. americanum* Hultén and St. John, is distributed in western North America between California and Alaska (Hultén & St. John, 1931). Fossil evidence substantiating a more extensive distribution for araceous plants has been found in Spitsbergen (presumably as imprints) referable to extant species. An aquatic aroid from the Tertiary flora, *Acorus brachystachys,* has been described recently by Schloemer-Jager from the Brogger Peninsula of the same island (Andrews, 1961).

Cockerell (1926) gives evidence supporting a once more westerly distribution of *Orontium* in North America. The relevant specimen consisted of a spadix, incomplete at the distal, fertile end, and its sterile stalk. Although the identity of the specimen is uncertain, it was sufficiently characteristic, according to the author, to be referred to this genus. The only character found to separate the fossil plant from the modern species was a lack of thickening of the scape below the spadix. Cockerell states that "this fossil may take the name *Orontium fossile* n. sp.; it adds one more to the numerous examples of genera now existing in the Eastern and Southern states, but found in the Rocky Mountain Region only in the fossil state."

If *Orontium* and related genera migrated from the region of the Arcto-Tertiary flora, then they were probably distributed over a wide range during the late Cretaceous and early Tertiary, from the east coast of the continent to the region of Colorado or perhaps even further west, under the influence of the equable climate of the era. At that period, the Appalachian Uplands were essentially a peneplain, affording ideal conditions for coastal-plain types such as *Orontium* in the streams and swampy areas. Uplift of this old Cretaceous (Schooley) peneplain during the Miocene epoch, and the accompanying climatic changes and erosion cycles resulted in greatly altered conditions. The climate to the west became much more arid, eliminating possible populations of *Orontium* west of the Mississippi River. A more restricted distribution was placed upon the species in the Appalachian Region and only relict populations survived in the favorable mesophytic and hydrophytic habitats of the newly elevated highlands. With the later recession of the sea, the newly-emergent Coastal Plain became available to the migrating species from the uplands, which found optimum conditions in the bog-marsh habitats. An account of the Coastal Plain element in the flora of the Appalachian uplands, correlated with physiographic history of the provinces concerned, has been described thoroughly by Kearney (1900), Harshberger (1903), Fernald (1931, 1937), Braun (1937), Carr (1938), Core (1938), *et al.*

Observation of the map (Fig. 1) shows that the present range of *Orontium* has extended into the formerly glaciated region in some of the Northeast. Peattie (1922) reports the occurrence of *Orontium* from as far inside the glacial boundary as Lake Oneida, New York. During the glacial and interglacial stages of the Pleistocene, the climatic fluctuations probably caused a retreat of the species to refugia farther south of the advancing ice. From these refugia, when the ice receded for the final time, probably it migrated to its present northernmost limits.

As stated earlier, varying chromosome numbers for *Orontium* have been reported. Huttleston (1953) has counted 28 somatic chromosomes from root tip squashes of four plants, two from Duval County, Florida, and two from Middlesex County, New Jersey. Both of these locations are in the Atlantic Coastal Plain. A different count, of 24 chromosomes, has been reported by Malvesin-Fabre (1945) in a thesis submitted to the University of Bordeaux, France. As this work was unobtainable, the source and the state of the studied plant material are not known to the present writer. However, the possibility of different numbers suggested that some correlation might exist between chromosome number and geographical distribution.

It was planned originally to work with meiotic material, but flowers from plants collected in early April of 1961

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Fig. 2. Chromosomes of *Orontium*. First meiotic metaphase. Material from Fentress County, Tennessee. × approx. 500.

had already undergone meiosis. In collections made in early September of the same year, meiosis had already occurred in young flowers that would not make their appearance until the following spring. It appears that the greater part of the meiotic process probably occurs sometime during the summer season, more than eight months in advance of of anthesis the following spring.

Only one determination of chromosome number was made from meiotic material. This was obtained quite by chance from the tip of an inflorescence collected in Fentress County, Tennessee, April 1961, in which meiosis was complete. A few pollen mother cells of the young sporogenous tissue showed 26 chromosomes at the first meiotic metaphase, the number not yet having been reduced. The inflorescence was collected in Newcomer's solution and the squash was made using acetocarmine. Some of the chromosomes exhibited a tendency to stick together, as evidenced by strand-like connections between them (Fig. 2). Because of the uncertainty relating to the exact time of meiosis, all other work was done with root tip chromosomes.

Living samples of *Orontium*, variously consisting of fruits, seedlings and mature plants, were obtained from various sources for root tip material. The samples were maintained in the green house in concrete water tanks during the course of investigation. Fresh, vigorously-growing root tips about 1 cm. in diameter were excised immediately before treatment.

The live tips were pretreated with α-bromonapthalene to inhibit spindle formation, shrink the chromosomes and render the centromeres more evident. The method of Tijo and Levan (1950), utilizing 8-hydroxyquinoline as a pretreatment agent, proved less effective in fixing the metaphase than α-bromonapthalene even though other conditions were constant. The root tips were then killed and fixed with alcohol-acetic acid (3:1) and stained with aceto-orcein after the method of LaCour (1941). The meristematic tip of the root was then excised and squashed in a drop of 45% acetic acid. Mitotic chromosome counts were obtained from 24 different populations and in every instance the diploid number was 26. Neither of the previosly reported numbers ($2n = 24, 28$) was ever found, although attempts to verify Huttleston's Duval County report were made using material collected from the same site. The chromosomes are very large, intensely-staining and easily observed where the squash preparation is good. The position of the majority of the centromeres is median (iso-brachial), a feature regarded by some workers (e.g. Levitzky, 1931) as representing the generalized or primitive condition. The chromosomes of one pair have clearly discernable satellites (Fig. 3). A tendency toward fragmentation at the centromere may account for Huttleston's report of $2n = 28$. However, this was a random occurrence in the present study.

Because the chromosome number appeared the same in all plants examined, regardless of source, consideration was given karyotypic variation among populations. Graphic portrayal of chromosome arm length was attempted, but, because of differential shrinkage and swelling of the chromosome arms it was not possible to match homologous chromosomes. Therefore, no correlation was found between quantitative chromosomal data and the geographical source of the samples studied. The only two chromosomes that could be paired with any degree of certainty were the two

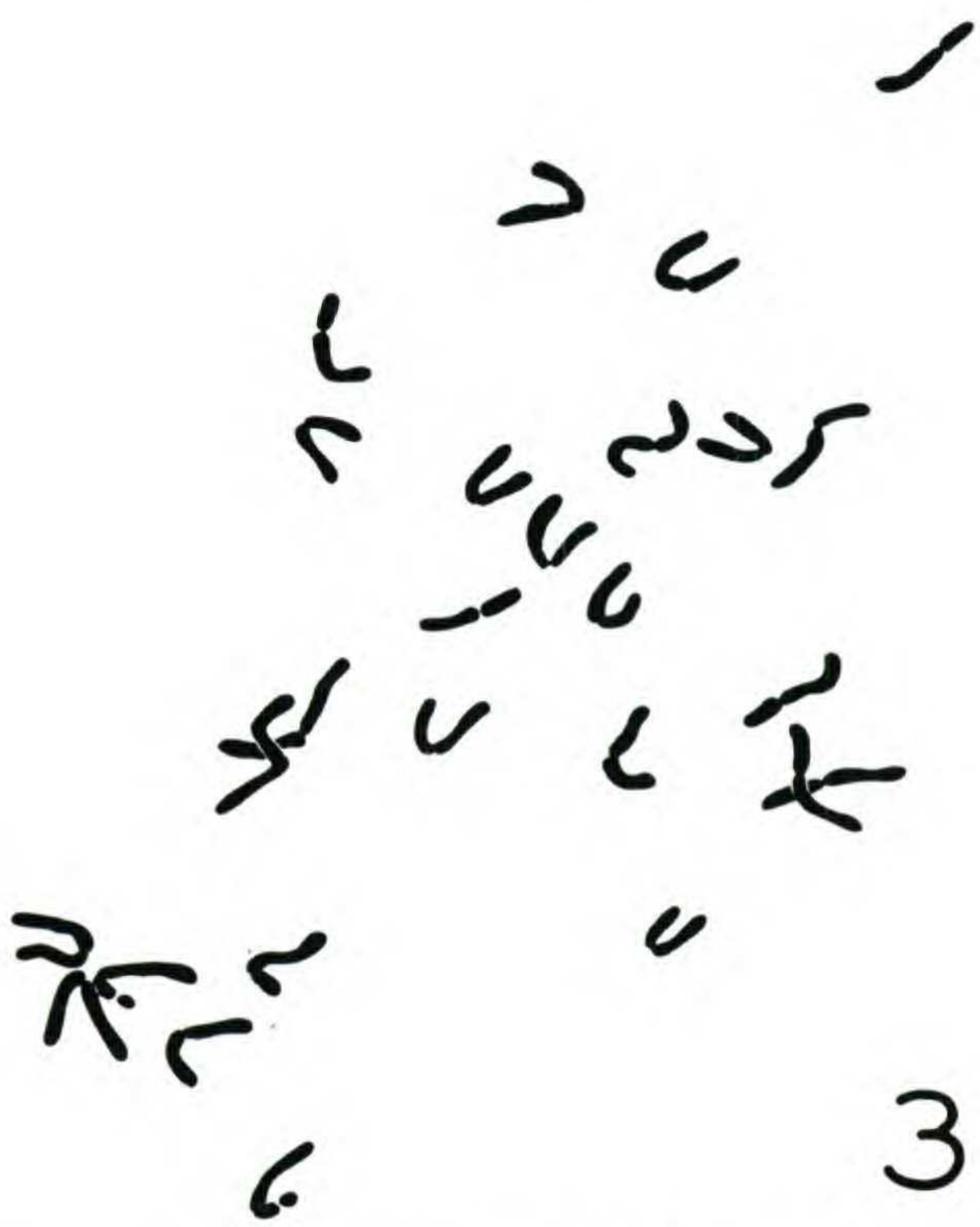

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Fig. 3. Chromosomes of *Orontium*. Mitotic chromosomes. Material from Cameron Parish, Louisiana. × approx. 700.

with satellites. Until a more reliable procedure is developed, permitting assessment of differences in linear dimensions, the quantitative information must be considered insignificant.

It appears that *Orontium* chromosomes would be particularly suitable for differential staining (see Fukuda and Kozuka, 1958, for work on *Trillium*), and that the homologous pairs of chromosomes might thereby be identified. There was some indication of differential stainability of the chromosomes of *Orontium* during the present investigation and this suggests the possibility of relating the populations to distribution.

SUMMARY

Information obtained from this study contributes to knowledge of the cytology of *Orontium*, a monotypic genus of the Araceae. The diploid chromosome number of all populations studied is 26, irrespective of geographical

source. Two satellites or secondary constrictions are evident in every karyotype and the chromosomes are extremely large (ca. 20μ), exhibiting essentially median centromeres.

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